

KOMBUCHA AS A NATURAL SOURCE OF HIGH PURITY

BACTERIAL CELLULOSE PRODUCTION

A RESEARCH PROJECT

Submitted by

PRITI A. TANGADE

RASIKA S. BHOSLE

SHARVARI S. PATIL

UNDER THE GUIDANCE OF

MS. MITALI NADKARNI

(Assistant Professor)

DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2025-2026

“Dissemination of education for Knowledge, Science and culture”

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

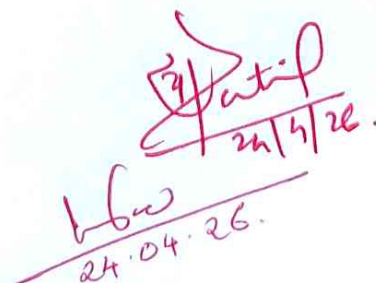
CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **PRITI A. TANGADE** studying in M. Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **“KOMBUCHA AS A NATURAL SOURCE OF HIGH PURITY BACTERIAL CELLULOSE PRODUCTION”** during academic year 2025-26.



Ms. Mitali Nadkarni

Research Project Guide



Examiner



Dr. T.C. Gaupale

Head of the Department
I/C Head
Department of Microbiology
Vivekanand College, Kolhapur
(Empowered Autonomous)

“Dissemination of education for Knowledge, Science and culture”

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **RASIKA S. BHOSLE** studying in M. Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **“KOMBUCHA AS A NATURAL SOURCE OF HIGH PURITY BACTERIAL CELLULOSE PRODUCTION”** during academic year 2025-26.

Ms. Mitali Nadkarni

Research Project Guide

Examiner

Dr. T.C. Gaupale

Head of the Department
I/C Head

Department of Microbiology
Vivekanand College, Kolhapur
(Empowered Autonomous)

“Dissemination of education for Knowledge, Science and culture”

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

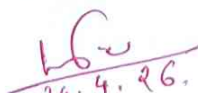
CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **SHARVARI S.PATIL** studying in M. Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **“KOMBUCHA AS A NATURAL SOURCE OF HIGH PURITY BACTERIAL CELLULOSE PRODUCTION”** during academic year 2025-26.



Ms. Mitali Nadkarni

Research Project Guide


24.4.26.
Examiner

Dr. T.C. Gaupale

Head of the Department
I/C Head
Department of Microbiology
Vivekanand College, Kolhapur
(Empowered Autonomous)

ACKNOWLEDGEMENT

I wish to express my deep sense of appreciation to Ms. Mitali Nadkarni , Assistant Professor, Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for her valuable support and expert guidance during the course of this study. She has been extremely understanding and cooperative and has always taken great interest in this work.

I wish to express my sincere thanks to Dr. C. B. Patil, Principal, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) and Dr. T. C. Gaupale, Head, Department of Microbiology for providing the laboratory facilities in the department to carry out the experimental work.

I express my thanks to Ms. V. V. Misal, Coordinator of PG Department of Microbiology and faculty members, Dr. S. D. Mali, Dr. K.K. Bhise, Ms. S. S. Shaikh, Ms. A. T. Patil, and Ms. P. P. Patil for their valuable suggestions and help during the work.

I convey my gratitude to Mrs. S. N. Salokhe (Laboratory Assistant), Mr. S. K. Maskar and Mr. D. R. Patil (Laboratory Staff) of the department for their kind help in the laboratory.

I am thankful to the librarian and library staff for providing facilities of computer and reference books. My special thanks and gratitude to my entire classmates who have been constant source of inspiration and help during entire project work. I am highly obliged to authors past and present whose literature has been cited.

Finally, I thank my family members for their blessings and support because of which this work has proved satisfactory to m

Place: Kolhapur

Date: 15/04/2026

Ms. Priti Anil Tangade *P. Tangade*
Ms. Rasika Sanjay Bhosale *R. Bhosale*
Ms. Sharvari Subhash Patil *S. Patil*

INDEX

Sr. No.	Title	Page No.
1.	Introduction	1-5
2.	Review of Literature	6-17
3.	Objectives	18-19
4.	Material and methods	20-28
5.	Result and Discussion	29-42
6.	Conclusion	43-44
7.	Bibliography	45-50

Introduction

Introduction

Trees play a vital role in maintaining the environment and supporting human life. Through photosynthesis, they produce oxygen, which is essential for all living beings. Moreover, they help mitigate greenhouse gas emissions by absorbing carbon dioxide. Trees also support biodiversity by providing food and shelter to a wide range of wildlife and humans. Overall, trees are crucial for maintaining ecological balance, preserving wildlife habitats and sustaining life on Earth.

In addition to their environmental significance, trees are a primary source of raw material for paper production. They contain cellulose fibers, an essential component that contributes to the structural integrity of paper. The paper industry is a significant part of the global economy, producing a wide range of products for various applications. With rapid industrialization and urbanization, paper consumption has steadily increased in high-income countries such as the United States, many nations in the European Union, and Japan (FAO, 2020).

To address the growing demand for paper while reducing deforestation, alternative sources such as agricultural residues and weed plant residues are being explored. These can serve the needs of the packaging industry and hygiene-related products like paper towels, toilet paper and disposable wipes (IPCC, 2021; WWF, 2023). Another sustainable alternative gaining attention is bacterial cellulose, which can serve as a replacement for tree-derived cellulose.

Bacterial cellulose (BC) is emerging as a superior substitute for plant-based cellulose due to its higher purity, better mechanical properties and environmentally friendly production process. Unlike plant cellulose, which requires chemical treatments to remove lignin and hemicellulose, the major bacterial families known to produce bacterial cellulose are: *Acetobacteraceae*, *Rhizobiaceae*, *Alcaligenaceae* thus eliminating the need for intensive post processing [1].

The nanostructure of bacterial cellulose plays a key role in determining its physical and mechanical properties. Its structure can be manipulated based on cultivation methods, types of bioreactors and whether the cellulose is obtained in a hydrated or freeze-dried state. The design of the growth medium is crucial, as microorganisms respond to environmental changes by altering protein synthesis and cell morphology. Compared to plant-derived cellulose, BC's thin nanofiber-based

3D network enhances its surface-area-to-volume ratio, enabling strong interactions with adjacent components [3].

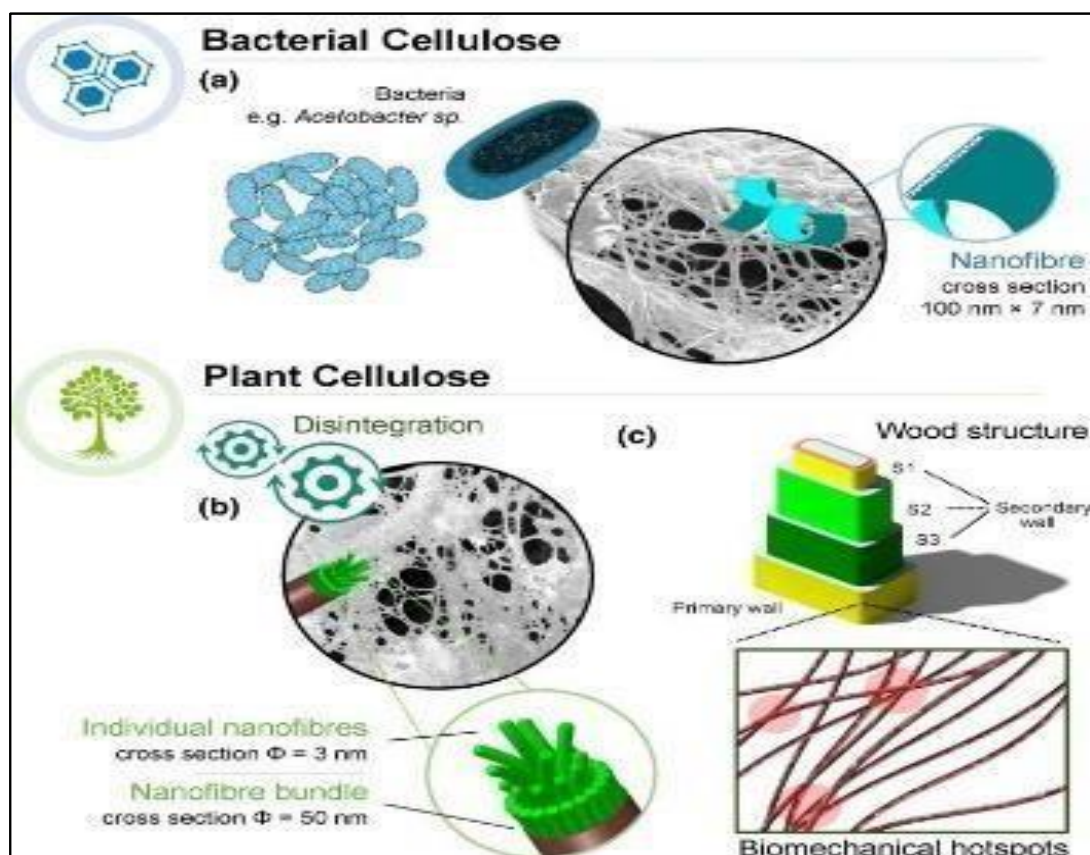


Fig 1: Comparison of bacterial cellulose and plant cellulose structure [2].

BC also exhibits excellent mechanical strength, a high degree of polymerization and crystallinity (approximately 90%), as well as a remarkable capacity to retain water. Its inherent purity free from lignin, hemicelluloses and pectin combined with its non-toxicity and biodegradability, makes it particularly attractive for medical applications, such as wound dressings and tissue engineering scaffolds [4]. Properties such as tensile strength, crystallinity, polymerization degree and moisture absorption capacity can be tailored by adjusting culture conditions, microorganism strains and the composition of the growth medium [5].

Kombucha serves as a natural and sustainable source for bacterial cellulose (BC) production due to its unique symbiotic culture of bacteria and yeast (SCOBY), which creates an ideal environment for cellulose synthesis. In this system, acetic acid bacteria, particularly *Komagataeibacter xylinus*, produce high-quality cellulose

at the air–liquid interface [6]. The fermentation process is economical and simple, requiring only basic ingredients such as tea and sugar, making it suitable for both laboratory and large-scale production. Additionally, the mutual interaction between bacteria and yeast within the kombucha matrix ensures stable fermentation and consistent cellulose yield and purity [7].

Cellulose yield in kombucha fermentation is influenced by several factors. Optimal production occurs with 5–10% sucrose as the carbon source and a suitable tea type providing essential polyphenols and nutrients. The ideal fermentation temperature is 28–30°C, under aerobic conditions to support bacterial cellulose synthesis. The process typically lasts 10–14 days, after which nutrient depletion and acid buildup reduces yield. An initial pH of 5.0–5.5 favors growth, though it gradually drops to around pH 3 as fermentation progresses. Finally, a well-balanced SCOBY (Symbiotic Culture of bacteria and yeast) with active bacteria and yeasts ensures efficient conversion of sugars and ethanol into cellulose [6, 7, 8, 10].

Bacterial cellulose is synthesized extracellularly by various Gram-negative bacteria, including those from the genera *Acetobacter*, *Aerobacter*, *Gluconacetobacter*, *Azotobacter*, *Agrobacterium*, *Achromobacter*, *Sarcina*, *Rhizobium*, *Pseudomonas*, *Salmonella*, and *Alcaligenes*. Among these, *Gluconacetobacter* species are extensively researched due to their ease of cultivation and ability to utilize a wide range of carbon sources [11].

Gluconacetobacter is a dominant genus of acetic acid bacteria in kombucha fermentation, primarily responsible for bacterial cellulose (BC) production. According to [7], *Gluconacetobacter xylinus* forms a cellulose pellicle at the air–liquid interface, which provides structural stability to the SCOBY (Symbiotic culture of bacteria and yeast) and supports microbial attachment. These bacteria oxidize ethanol, produced by yeasts, into acetic and gluconic acids, thereby lowering the pH and contributing to the beverage’s antimicrobial properties [8]. Their metabolic activity facilitates a symbiotic relationship with yeasts, ensuring continuous and balanced fermentation. [9] also identified *Gluconacetobacter* as a core bacterial genus consistently present in diverse kombucha cultures. Overall, this genus is vital for acid production, cellulose synthesis and maintaining the microbial integrity and texture of kombucha.

Applications

Paper industry: The pulp and paper industry is another field with immense potential for the application of bacterial cellulose. Owing to its composition of tiny cellulose micro fibril clusters, Bacterial cellulose has been utilized as binder in paper manufacturing, which greatly enhances the strength and durability of the resulting pulp. This makes bacterial cellulose particularly valuable for producing special high strength paper required for long life currency and archival document restoration [12].

1. Reinforcement in paper and packaging
2. Sustainable textile and Leather alternatives
3. Biocomposites and Electronics

Food Industry: Since 1992, the U.S. Food and Drug Administration (FDA) has classified bacterial cellulose (BC) as “generally recognized as safe” (GRAS) due to its nature as a dietary fiber. Owing to its high purity, ease of production and ability to form diverse textures and morphologies — such as particles, spheres, filaments, pulps, films and whiskers — BC demonstrates remarkable versatility for use in food application [12].

1. Dietary fibres and Low calorie foods
2. Edible films and Food additives

Medical and Biomedical Industry: The growing demand for bio-based materials has brought significant attention to bacterial cellulose (BC) for its immediate applications in biomedical fields, including wound healing, tissue engineering and drug delivery. BC accelerates wound repair by promoting re-epithelialization through enhanced cellular adhesion, proliferation, migration and differentiation, making it highly suitable for regenerative medicine. In addition, BC is being explored for use in biomedical devices such as dental implants, artificial blood vessels, vascular grafts and implants for the urethra, nerves, cornea and retina [12].

1. Wound dressing and Artificial skin
2. Tissue engineering
3. Drug delivery systems



Review of Literature

Review of Literature

1. History:

The first report of bacterial cellulose (BC) was made by Adrian J. Brown in 1886 during acetic-acid fermentation studies, where he identified a gelatinous pellicle as pure cellulose [13]. Later, research identified cellulose-producing bacteria such as *Komagataeibacter* and established key production conditions like oxygen and carbon sources [13]. Advances in molecular biology revealed the cellulose synthase complex and its regulation (e.g., c-di-GMP), enabling better understanding and optimization of BC production [14]. Due to its high purity, nanofibrillar structure, strength, and water-holding capacity, BC is widely used in biomedical applications and foods like nata de coco [15][16]. BC is synthesized extracellularly by polymerization of UDP-glucose into β -1,4-linked glucan chains, forming a highly crystalline, three-dimensional network. Unlike plant cellulose, it lacks lignin and hemicellulose, making it highly pure and suitable for industrial and medical use [17][18][19].

2. Bacterial Cellulose: Structure and Role

Bacterial cellulose (BC) is a unique biopolymer synthesized by certain species of acetic acid bacteria, primarily *Komagataeibacter xylinus* (formerly *Gluconacetobacter xylinus*), *Acetobacter* and *Agrobacterium* species. Unlike plant-derived cellulose, BC is produced extracellularly as a pure form of cellulose composed solely of β -1,4-linked D-glucose chains. Because it lacks lignin, hemicelluloses and other plant impurities, BC offers exceptional purity, crystallinity and mechanical strength, making it suitable for biomedical, food and industrial application [12,23].

2.1 Comparison of Bacterial cellulose with plant derived cellulose:

Features	Plant Cellulose	Bacterial Cellulose
Source	Plants [wood, cotton]	Bacteria like <i>Komagataeibactor</i>
Purity	Contains lignin and Hemicellulose	Highly pure [no lignin]
Structure	Less organized fibres	Nano- sized fibriller network
Crystallinity	Moderate	High.
Water holding capacity	Low	Very High [>90 %]
Strength	Good	Very high [strong and flexible]
Processing	Requires heavy chemical treatment	Requires mild purification .
Biocompatibility	Moderate	Excellent[used in medical fields]

Table No 1: Difference between Bacterial cellulose and Plant cellulose [15,20, 21,22].

2.2 Structural Organization of Bacterial Cellulose

2.2.1 Molecular Structure:

At the molecular level, BC is composed of linear β -1,4-D-glucopyranose units forming long glucan chains, identical in chemical composition to plant cellulose [24]. These chains associate through extensive hydrogen bonding to form elementary fibrils, which further aggregate into micro fibrils exhibiting a high degree of crystallinity (80–90%) [23, 26].

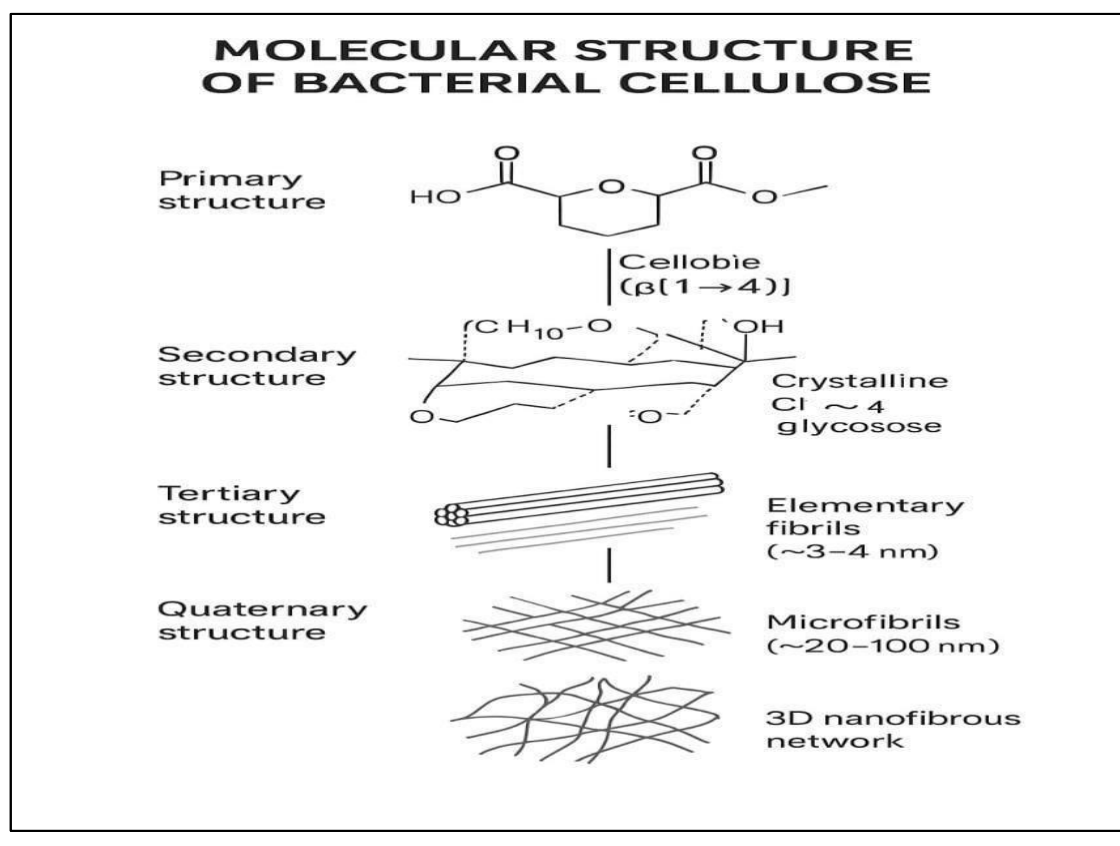


Fig 2: Molecular structure of bacterial cellulose [25].

2.2.2 Super molecular and Nanofibrillar Structure:

BC fibers have diameters ranging from 20 to 100 nm, forming a three-dimensional nanofibrillar network. This network provides BC with a high surface area, porosity, and tensile strength [1]. The nanofibrillar arrangement also enables strong water-holding capacity, as the spaces between fibrils can retain large amounts of water, contributing to the hydrogel-like nature of BC pellicles [24].

2.2.3 Purity and Chemical Composition:

Unlike plant cellulose, BC is inherently free from lignin, hemicellulose, and pectin. This high purity eliminates the need for chemical purification steps required in plant cellulose extraction and enhances its biocompatibility and biodegradability, which are essential for biomedical applications [23].

2.3 Biosynthesis of Bacterial Cellulose

BC is synthesized by bacteria through the polymerization of glucose residues derived from UDP-glucose, catalyzed by cellulose synthase complexes encoded by *bcs* genes [27]. The biosynthesis involves:

- i. Polymerization of glucose into β -1,4-glucan chains.
- ii. Crystallization of glucan chains into micro fibrils at the cell surface.
- iii. Assembly of fibrils into a pellicle at the air–liquid interface [23].

Environmental factors such as carbon source, oxygen level, temperature, and pH influence the yield and structural quality of BC [12].

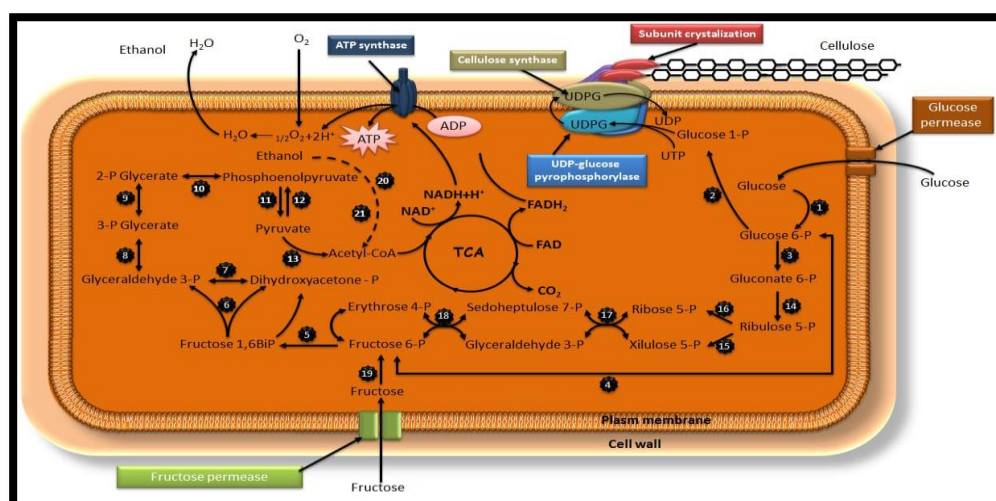


Fig No 3: Biosynthesis pathway of bacterial cellulose

3. Kombucha Tea as Source of Bacterial cellulose

Kombucha tea, popularly known as the “tea of immortality” or “elixir of life,” is a traditional fermented beverage produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY). This naturally carbonated drink is gaining global attention for its refreshing flavor, probiotic microorganisms, and potential health benefits such as detoxification, antioxidant activity, and improved gut health [7].

3.1 Historical Background of Kombucha Tea:

The origin of Kombucha tea is ancient and somewhat disputed. Most historical evidence traces its beginnings to Northeast China (Manchuria) around 220 B.C., during the Tsin Dynasty, where it was consumed for its energizing and healing properties [30]. The name Kombucha is believed to have originated from a Korean physician named Dr. Kombu, who brought the tea to Japan to treat Emperor Ingyō around 414 A.D. The word “Kombu-cha” translates to Kombu’ s tea,” with cha meaning tea in Japanese [29] . Following its use in East Asia, Kombucha spread to Japan and subsequently to Russia and Eastern Europe via trade routes and cultural exchanges. In Russia, it became well known as “Tea Kvass” in the early 20th century. Its popularity expanded into Germany and other European countries during the 1920s and 1930s [7,8]. The beverage’s consumption declined during World War II due to shortages of tea and sugar but was later revived. By the late 20th and early 21st centuries, Kombucha gained recognition in Western countries, particularly in the United States, as a functional probiotic beverage [10,30].

3.2. Description and Composition:

Kombucha is prepared by fermenting sweetened black or green tea using a SCOBY, a symbiotic culture consisting of bacteria and yeast. During fermentation, the SCOBY forms a gelatinous cellulose biofilm on the surface of the liquid [7]. This biofilm, composed mainly of bacterial cellulose, is primarily produced by *Komagataeibacter xylinus* (previously *Gluconacetobacter xylinus*) [6]. In the fermentation process, the yeasts in the SCOBY (symbiotic culture of bacteria and yeast) such as *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*—hydrolyze sucrose into glucose and fructose, converting a portion into ethanol. The acetic acid bacteria (AAB) component then oxidizes ethanol into acetic acid, along with the production of gluconic and glucuronic acids [8,10]. These biochemical transformations are responsible for the beverage’s acidic taste, low pH and detoxifying potential.

4. Cellulose Production Process in Kombucha Fermentation

In Kombucha fermentation, the synthesis of bacterial cellulose (BC) represents a vital biochemical event driven by specific bacteria within the symbiotic culture of bacteria and yeast (SCOBY). The cellulose network forms the distinctive

gelatinous pellicle observed on the surface of the fermenting tea. This biopolymer provides structural support to the microbial community and significantly influences the overall stability and quality of the fermentation process [6, 7].

4.1 Role of Microorganisms in Cellulose Formation

Within the SCOBY (Symbiotic culture of bacteria and yeast) the bacterial component particularly *Komagataeibacter xylinus* (formerly known as *Gluconacetobacter xylinus*) is primarily responsible for the biosynthesis of cellulose. These acetic acid bacteria (AAB) metabolize carbon sources such as sucrose and its hydrolysis products (glucose and fructose) to produce bacterial cellulose (BC) alongside organic acids, including acetic, gluconic and glucuronic acids [6,10].

Simultaneously, yeast species such as *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Brettanomyces bruxellensis* initiate fermentation by breaking down sucrose into ethanol and carbon dioxide. The generated ethanol then serves as a substrate for acetic acid bacteria, which further oxidize it into acetic acid while synthesizing cellulose fibers. These fibers aggregate to form the pellicle at the liquid–air interface, where aerobic conditions favor AAB metabolism[8].

5. Production Methods of Bacterial Cellulose from Kombucha Tea

Bacterial cellulose (BC) production in Kombucha is a result of symbiotic fermentation between acetic acid bacteria (AAB) and yeasts. The bacteria, mainly *Komagataeibacter xylinus* are responsible for cellulose synthesis, while the yeasts initiate sugar hydrolysis and ethanol formation. During fermentation, the microorganisms interact dynamically transforming the sweetened tea into a slightly acidic, effervescent beverage, with the cellulose pellicle forming at the air–liquid interface. This process is aerobic and thus oxygen availability is essential for efficient cellulose biosynthesis [6, 8].

5.1 Step-by-Step Production Process:

5.1.1 Preparation of the Culture Medium:

The substrate for BC production consists of black or green tea infusion sweetened with sucrose (50–100 g/L). Tea provides essential nutrients such as

polyphenols, nitrogenous compounds, and minerals that support microbial metabolism [7]. The tea is boiled for 5–10 minutes to extract soluble compounds and sterilize the medium. After cooling to room temperature, sugar is added, and the medium is adjusted to an initial pH of 5.0–5.5 before inoculation.

5.1.2 Inoculation with SCOBY (symbiotic culture of bacteria and yeast):

The inoculum includes a portion of fermented Kombucha broth (10–20% v/v) and a fragment of the cellulose pellicle (SCOBY). This ensures the introduction of active bacterial and yeast populations necessary for balanced fermentation [6].

5.1.3 Fermentation Phase:

The inoculated medium is incubated in wide-mouth glass or stainless-steel containers under static, aerobic conditions at 25–30°C for 7–14 days. Yeast metabolism: Yeasts such as *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* hydrolyze sucrose into glucose and fructose using invertase enzymes and produce ethanol and Carbon dioxide through glycolysis.

Bacterial metabolism: *Komagataeibacter xylinus* utilizes glucose and ethanol to produce acetic acid, gluconic acid, and bacterial cellulose. The bacteria secrete cellulose Microfibrils through pores in their cell walls, forming a continuous pellicle at the surface [10].

5.1.4 Cellulose Pellicle Development:

Over time, the bacterial cellulose thickens to form a gelatinous mat or “mother”, floating on the surface due to its fibrous and porous structure. The cellulose acts as a protective biofilm, maintaining aerobic conditions for the bacterial community and stabilizing the pH by trapping organic acids within its matrix [8].

5.1.5 Harvesting and Purification:

After the desired fermentation period, the cellulose mat is carefully removed and subjected to purification:

- It is washed several times with distilled water to remove tea pigments and residual acids.

- It is then treated with 1–2% NaOH or boiled sodium carbonate solution at 80–90°C for 30–60 minutes to eliminate microbial cells and proteins.
- Finally, the purified cellulose is rinsed to neutral pH and either dried for storage or used wet for experimental applications [7].

5.1.6 Drying and Post-processing:

The wet bacterial cellulose (containing 95–98% water) may be air-dried, oven-dried, or freeze dried depending on the intended use. Drying influences its mechanical strength, transparency, and porosity—critical factors for biomedical or paper-making applications.

6. Overview of Bacterial Cellulose (BC) Production Methods:

The production of bacterial cellulose (BC) involves several fermentation approaches that differ in productivity, morphology control and scalability. The major methods include static culture, agitated culture and controlled bioreactor systems, along with recent advances such as low-cost substrate utilization and improved downstream processing [15,17].

6.1 Static (Surface) Culture :

In this traditional method, BC producing bacteria such as *Komagataeibacter xylinus* or *Gluconacetobacter spp.* are incubated statically in shallow vessels under aerobic conditions. The bacteria synthesize cellulose at the air–liquid interface, forming a thick, uniform pellicle or film [17].

Product: Highly crystalline, dense pellicles with superior mechanical strength.

Advantages: Simple operation, uniform film quality and easy control of product

Limitations: Low volumetric productivity, long cultivation time and poor scalability for industrial application.

6.2 Agitated (Shaken or Stirred) Culture

In agitated systems, cultures are continuously mixed in flasks or stirred-tank reactors. Agitation enhances oxygen transfer but changes the morphology of BC, yielding fibrous aggregates, pellets, or irregular fragments instead of cohesive pellicles [15].

Advantages: Higher productivity, faster cellulose synthesis, and suitability for large-scale liquid operations.

Limitations: Shear stress can disrupt fibril formation, reduce crystallinity, and may lead to the emergence of non-producing mutants. Material properties also differ from those of static pellicles.

6.3 Bioreactor Systems (Controlled Fermentation Reactors)

To overcome the limitations of conventional cultures, various bioreactor configurations—including airlift, rotating-disk, membrane, and fed-batch reactors—have been developed. These systems maintain controlled oxygen transfer, pH, temperature, and nutrient feeding, improving productivity and allowing morphology tailoring [18].

Advantages: Enhanced process control, greater yield, and potential scalability for industrial production.

Limitations: Complex design, high operational cost, and challenges in maintaining uniform shear and oxygen distribution.

7. Qualitative Characterization of Bacterial Cellulose via Acid Hydrolysis and Schultze's Reagent Test

7.1 Acid Hydrolysis Test:

Cellulose is a polysaccharide composed of β -1,4-linked glucose units, which can be broken down into glucose by acid hydrolysis. In this test, the cellulose sample is treated with dilute acid (H_2SO_4 or HCl) and heated, causing hydrolysis of glycosidic bonds. The resulting glucose is then detected using Benedict's reagent, which produces a brick-red precipitate due to the reduction of Cu^{2+} ions. This confirms the presence of cellulose indirectly through the formation of reducing sugars [41,42].

7.2 Schultze's Reagent Test for Cellulose:

The iodine test is used to differentiate cellulose from starch based on structural differences. When iodine solution (I_2 and KI) is added to cellulose, no color change occurs because cellulose lacks the helical structure required to form a colored complex with iodine. After addition of Zinc chloride solution colour change to the blue colour and it is considered as positive test for Cellulose.[43,44] .

8. Consistency and Quality Control Challenges

Maintaining uniform BC quality across different production batches remains a critical issue that limits commercialization and regulatory acceptance.

8.1 Biological variability:

BC yield and quality are highly sensitive to bacterial strain, carbon source, pH, temperature, and oxygen levels. Small changes can affect fiber diameter, crystallinity and tensile strength [34].

8.2 Lack of standardization:

There is no universal standard for BC quality assessment. Variations in parameters such as degree of polymerization, crystallinity index, tensile strength and porosity exist among producers [35].

8.3 Contamination risks:

Open fermentation systems, like Kombucha, are prone to microbial contamination by molds or unwanted yeasts, leading to inconsistent color, odor and product safety [36].

9. Purification Challenges

Bacterial cellulose is secreted extracellularly as a hydrated pellicle that contains bacterial cells, medium residues, and metabolic by-products. Achieving high-purity BC suitable for industrial and biomedical applications requires extensive purification steps.

9.1 Complex purification requirements:

Alkaline treatment using NaOH or KOH at high temperatures is commonly used to remove bacterial cells and impurities. However, this process may cause partial degradation or decrystallization of cellulose fibrils, reducing quality [37].

9.2 Environmental concerns:

The alkaline purification process is energy- and resource-intensive and generates wastewater containing cell debris and alkali residues, posing environmental disposal challenges [38].

9.3. Structural damage risk:

Harsh chemical treatments can disrupt BC's nanofibrillar 3D network, leading to loss of porosity, reduced water-holding capacity and weaker mechanical properties [39].

9.4. Alternative green methods:

Eco-friendly approaches involving enzymatic digestion (cellulase, protease), mild acid treatments, ultrasonication and supercritical CO₂ extraction are being explored, though they remain costly and less scalable [40].

10. Future Aspects of Kombucha Derived Bacterial Cellulose :

- Genetic engineering of cellulose producing bacteria for higher yield and enhanced properties.
- Creation of Bacterial cellulose based composites for flexible electronics, energy storage and sensors.
- Use of Bacterial Cellulose in food technology as edible films, coatings and functional dietary fibers.
- Development of functionalized Bacterial Cellulose materials for advanced biomedical applications.



Objective

Objectives:

- To understand its physical properties and surface microflora of Kombucha Tea.
- To isolate and identify *Komagataeibacter xylinus* bacteria from Kombucha Tea.
- To obtain high purity bacterial cellulose by cultivating SCOBY in specialized enrichment broth.
- To validate the identity and purity of extracted cellulose through iodine staining and acid hydrolysis test.

A decorative horizontal scroll graphic with a black outline and rounded ends. The scroll is slightly curved, giving it a three-dimensional appearance. The title "Materials and Methods" is centered within the scroll in a bold, black, serif font.

Materials and Methods

Materials and Methods:

1. Collection of Kombucha Tea

Kombucha tea was bought online from Toyo Company. The bottle was received in sealed conditions and used as starter culture.

➤ **Ingredients:**

- Kombucha culture
- Filtered water
- Cane sugar
- Organic Green tea
- Organic Black tea

➤ **Nutritional Facts:**

Components	Approximate Amount
Calories	26.72 kcal
Protein	0.67gm
Total carbohydrates	6.01gm
Sugar	5.28gm
Fat	0gm

2. Kombucha Fermentation Process:

➤ **Materials:**

- Green tea
- Kombucha tea
- Sugar
- Autoclave
- Flask
- Cotton

Method:

- i Sweetened tea medium was prepared by dissolving sucrose (30gm) in distilled water.
- ii Then green tea bags were added and mixture was boiled for 10-15 minutes.
- iii Then the solution was filtered using strainer.
- iv In the next step the prepared sweet tea was sterilized using an autoclave at 121⁰C for 15 minutes.
- v After sterilization, the medium was allowed to cooled to room temperature.
- vi After cooling, Kombucha tea from Toyo Company was added aseptically into the sterile sweet tea.
- vii Then the flask was kept for fermentation at 30 ⁰C for 8-10 days .

3. Inoculation of Fermented sweet tea in HS (Hestrin -Schramm) Broth (500ml):**➤ Materials:**

- Conical flask
- Measuring cylinder
- Autoclave
- Incubator

Components	Amount
Glucose	10gm
Peptone	2.5gm
Yeast extract	2.5gm
Disodium phosphate	1.35gm
Citric Acid	0.575gm
Distilled water	500 ml

Method:

- i 500 ml of distilled water was taken in conical flask.
- ii Glucose, peptone, yeast extract, disodium phosphate and citric acid were weighed accurately.
- iii All the ingredients were added to the distilled water mixed properly until completely dissolved.
- iv The medium was sterilized in autoclave for 15 minutes.
- v After sterilization, the medium was allowed to cool and used for cellulose production.
- vi Next fermented sweet tea was added in completely cooled HS broth.
- vii The flask was kept undisturbed at room temperature.
- viii After 3-7 days, a thin white layer started forming on the surface, which gradually becomes thicker.

4. Isolation of Micro flora from Kombucha Tea**➤ Materials:**

- Sterile Petri plates
- Fermented Kombucha media
- Incubator
- Glass slides and cover slip
- Staining reagents (Gram staining kit),
- Culture tubes
- Biochemical test media and reagents

➤ **HS Agar components:**

Components	Amount
Glucose	10gm
Peptone	2.5gm
Yeast extract	2.5gm
Disodium phosphate	1.35gm
Citric Acid	0.575gm
Agar-agar	7.5gm
Distilled water	500 ml

Methods:

i Isolation and Selection of Colonies:

Samples were cultured on HS agar plates and incubated under appropriate conditions (28–30°C for 24–48 hours). After incubation, morphologically distinct colonies were selected based on visible differences in size, shape, color, margin, elevation, and surface characteristics.

ii Morphological Characterization:

Colony morphology of each isolate was examined visually and recorded. Parameters such as colony form, elevation, margin, opacity, pigmentation, and texture were documented. Each selected colony was subcultured onto fresh HS medium to obtain pure cultures. The plates were incubated under the appropriate conditions (28–30°C for 24–48 hours).

iii Microscopic Examination:

Smears of each isolate were prepared on clean glass slides and subjected to Gram staining. The stained slides were observed under a microscope to determine cell morphology, Gram reaction, and cellular arrangement.

iv Biochemical Characterization:

Biochemical tests including Sucrose, Galactose, Lactose, Xylose, Glucose, Catalase and H₂S production tests, were performed following standard microbiological protocols. The results were recorded to assess the metabolic and enzymatic properties of the isolates.

5. Confirmation of Acid Producing Bacteria:

➤ Materials :

- Sterile Petri plates
- Wire loop
- Incubator
- Fermented Kombucha media

➤ GYC agar (Glucose yeast calcium carbonate) (200ml) :

Components	Amounts
Glucose	20gm
Yeast extract	2gm
Calcium carbonate	4gm
Agar-agar	3gm
Distilled water	200 ml

Method:

i Preparation of GYC Medium:

GYC agar medium was prepared according to standard composition and sterilized by autoclaving. The sterile medium was poured into Petri plates and allowed to solidify under aseptic conditions.

ii Inoculation of Isolates:

Previously isolated pure bacterial cultures were aseptically streaked onto the surface of GYC agar plates using a sterile inoculating loop.

iii Incubation:

The inoculated plates were incubated at 30°C for 24–48 hours under aerobic conditions.

iv Observation and Confirmation:

After incubation, plates were examined for the presence of clear zones (halos) surrounding the colonies. The formation of clear zones indicates the dissolution of calcium carbonate due to acid production.

6. Collection and Harvesting the Bacterial Cellulose layer:

➤ **Materials:**

- Fermented media containing bacterial cellulose layer
- Sterile forceps
- Distilled water
- Clean beaker
- Filter paper
- Petri plate

Method:

- i** After fermentation, a thick bacterial cellulose layer was observed on the surface of the fermented media.
- ii** The cellulose layer was carefully removed using sterile forceps.
- iii** The collected cellulose was washed several times with distilled water to remove residual medium and impurities.
- iv** The washed cellulose was placed on filter paper to remove excess water.
- v** Then it was collected in a clean Petri plate for further use.

7. Treatment of Bacterial Cellulose layer:

➤ Materials:

- Distilled water
- Hot plate
- Forceps
- Filter paper
- Petri plate

➤ Preparation of 0.1 M NaOH Solution(200ml):

- 0.8gm NaOH pellets were slowly added in 200 ml distilled water.
- Stir well until completely dissolved.

Methods:

- The collected bacterial cellulose layer was transferred into a beaker containing 0.1 M NaOH solution.
- The mixture was heated at about 80-90⁰ C for 30 minutes to remove bacterial cells and impurities.
- After heating, the cellulose was removed using forceps.
- The treated cellulose layer was washed several times with distilled water until the pH become 7.
- The purified cellulose was kept in distilled water for further analysis and some layer was kept in Petri plate for drying.

8. Bacterial Cellulose Confirmation Tests:

A. Acid Hydrolysis Test:

➤ Materials:

- Dried bacterial cellulose sample
- Dilute sulfuric acid (1–5% H₂SO₄)
- Distilled water
- Sodium hydroxide (NaOH)

- Test tubes
- Water bath
- Filter paper / centrifuge

➤ **Benedict's reagent or DNS reagent(100ml):**

Components	Amounts
Sodium citrate	17.3 gm
Sodium carbonate	10gm
Copper sulphate	1.73gm
Distilled water	100ml

Method:

- Take 0.5 g dried bacterial cellulose and hydrolyze it with dilute sulfuric acid by heating at 90–100°C for 15 minutes.
- After cooling, neutralize the solution with NaOH and filter to obtain a clear filtrate.
- Add Benedict's reagent to the filtrate and heat to test for reducing sugars.

B. Schultze's Reagent Test

➤ **Materials :**

- Bacterial cellulose sample
- Iodine solution ($I_2 + KI$)
- Zinc Chloride solution
- Test tubes / glass slide

Method:

- Place a small piece of bacterial cellulose on a slide or in a test tube.
- Add a few drops of iodine solution.
- Then wait for 2 minutes and then add 2-3 drops Zinc chloride solution.
- Observe the blue color change.

Results and Discussion

Results:

1. Preparation of Sweet tea:

The prepared tea solution appeared clear brown with characteristics aroma of tea. The pH of fresh prepared sweetened tea medium was approximately 5.5.-6.0



Fig No. 4: Prepared



Fig No. 5: Inoculated

2. Inoculation with Kombucha Tea:

After cooling, HS broth was inoculated with Fermented Kombucha tea.



Fig No 6: HS broth with fermented

Discussion:

The sweet tea (pH 5.5–6.0) provided a suitable medium for microbial growth. After inoculation with kombucha, fermentation was initiated, supporting cellulose-producing bacteria.

3. Fermentation process:

3.1 Early Fermentation stage:

After 2-3 days, small bubbles were observed in the liquid indicating microbial fermentation. The pH of medium was decreased from about 5.8 to around 4.5. A very thin transparent layer began forming air-liquid interface.



Fig No 7: Initial bacterial cellulose formation during early fermentation

3.2 Intermediate Fermentation Stage:

Between 4 and 7 days, the cellulose layer becomes thicker and more visible. The pellicle appeared whitish and slightly elastic. The pH further decreased to around 3.5- 4.0 and tea developed a slightly sour smell.



Fig No 8: Increased cellulose production and turbidity intermediate fermentation

3.3. Late Fermentation Stage:

After 8-10 days of fermentation, a thick layer of cellulose was clearly visible on the surface. The layer becomes firm and opaque white. The fermented liquid became more acidic with a pH of 3.



Fig No 9: Formation of thick, firm cellulose layer during prolonged fermentation stage

Discussion:

Early bubbles and pH drop indicate active fermentation and initial cellulose formation. Increased thickness in mid-stage shows higher bacterial activity. Final thick layer and low pH confirm maximum cellulose production.

4. Isolation and Characterization of Micro flora from Fermented Kombucha Tea Medium:

After incubation for 24-48 hours, five morphologically distinct colonies were observed on the HS agar plates. These colonies were selected and further analyzed based on colony characteristics, microscopic morphology and biochemical test.

Five colonies were designated as K1, K2, C1, C2, and C3.

4.1. K1:

- Colony Characteristics:

Colony characteristics of the isolated colony obtained from HS agar after incubation at room temperature (RT) for 48 hours.

Table No 1: Colony Characteristics of K1

Size	Shape	Colour	Margin
2mm	Circular	White	Entire

Surface	Elevation	Opacity	Consistency
Smooth	Low convex	Opaque	Sticky

- Morphological Characteristics :

Table no 2: Morphological Characteristics of K1

Gram Nature	Motility
Gram Negative Rod shaped	Non Motile

- Biochemical Testing:

Table no 3: Biochemical Testing of K1

Test	Acid	Gas
Sucrose	Negative	Positive
Galactose	Positive	Positive
Lactose	Negative	Negative
Maltose	Positive	Negative
Glucose	Positive	Positive
Catalyse	Positive	-
H ₂ S	positive	-

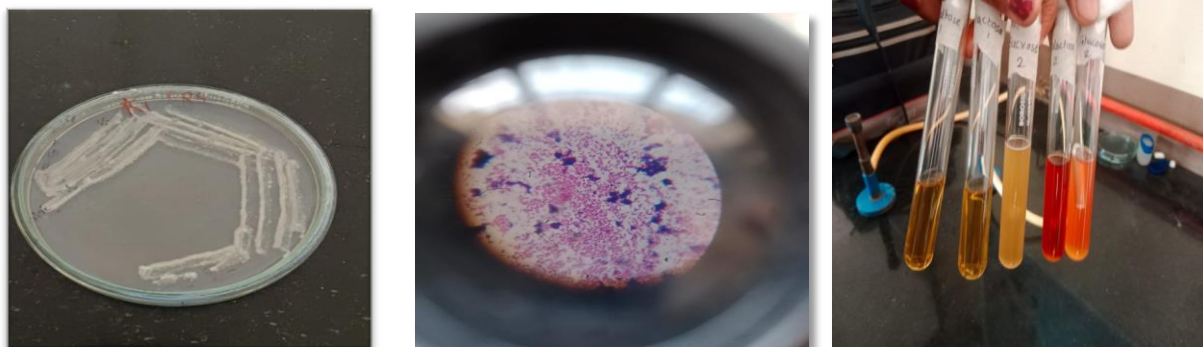


Fig No 10: Morphological (colony and microscopic) and biochemical characterization of the isolated bacterial strain K1.

4.2. K2:

- Colony Characteristics:

Colony characteristics of the isolated colony obtained from HS agar after incubation at room temperature (RT) for 48 hours.

Table No 4: Colony Characteristics of K2

Size	Shape	Colour	Margin
1mm	Circular	White	Irregular

Surface	Elevation	Opacity	Consistency
Smooth	Low convex	Opaque	Sticky

- Morphological Characteristics :

Table No 5: Morphological Characteristics of K2

Gram Nature	Motility
Gram positive cocci shaped	Non Motile

- Biochemical Testing

Table No 6: Biochemical Testing of K2

Test	Results	Gas
Sucrose	Negative	Positive
Galactose	Positive	Positive
Lactose	Positive	Positive
Xylose	Negative	Negative
Glucose	Positive	Positive
Catalase	Positive	-
H ₂ S	Postive	-

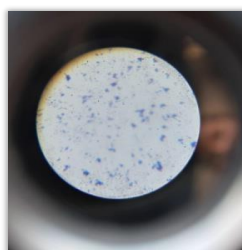


Fig No 11: Morphological (colony and microscopic) and biochemical characterization of the isolated bacterial strain K2.

4.3. C1:

- Colony characters:

Colony characteristics of the isolated colony obtained from HS agar after incubation at room temperature (RT) for 48 hours.

Table No 7: Colony Characteristics of C1

Size	Shape	Colour	Margin
1mm	Circular	White	Irregular

Surface	Elevation	Opacity	Consistency
Smooth	Low concave	Opaque	Sticky

- Morphological Characteristics:

Table No 8: Morphological Characteristics of C1

Gram Nature	Motility
Gram positive cocci shaped bacteria	Non Motile

- Biochemical Testing

Table No: 9. Biochemical Testing of C1

Test	Results	Gas
Sucrose	Negative	Positive
Galactose	Positive	Negative
Lactose	Positive	Positive
Xylose	Negative	Negative
Glucose	Positive	Negative
Catalase	Positive	-
H ₂ S	Postive	-

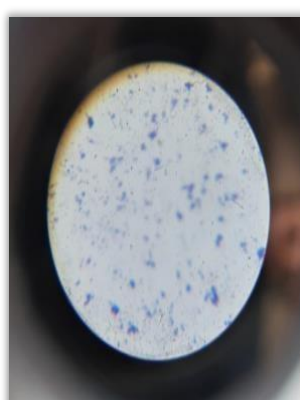


Fig No 12: Morphological (colony and microscopic) and biochemical characterization of the isolated bacterial strain C1.

4.4. C2:

- Colony Characteristics:

Colony characteristics of the isolated colony obtained from HS agar after incubation at room temperature (RT) for 48 hours.

Table No 10: Colony Characteristics of C2

Size	Shape	Colour	Margin
1mm	Circular	White	Entire

Surface	Elevation	Opacity	Consistency
Smooth	Low convex	Opaque	Sticky

- Morphological Characteristics :

Table No 11: Morphological Characteristics of C2

Gram Nature	Motility
Gram positive shaped cocci	Non Motile

- Biochemical Testing:

Table No 12: Biochemical Testing of C2

Test	Results	Gas
Sucrose	Negative	Negative
Galactose	Positive	Negative
Lactose	Positive	Negative
Xylose	Negative	Negative
Glucose	Positive	Negative
Catalase	Positive	-
H ₂ S	Postive	-

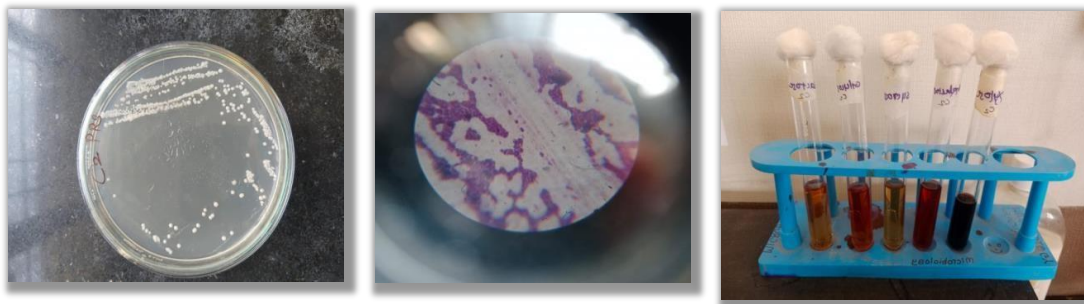


Fig No13: Morphological (colony and microscopic) and biochemical characterization of the isolated bacterial strain C2.

4.5. C3:

- Colony Characteristics:

Colony characteristics of the isolated colony obtained from HS agar after incubation at room temperature (RT) for 48 hours.

Table No 13: Colony Characteristics of C3

Size	Shape	Colour	Margin
1mm	Circular	White	Irregular

Surface	Elevation	Opacity	Consistency
Smooth	Low convex	Opaque	Sticky

- Morphological Characteristics :

Table No 14: Morphological Characteristics of C3

Gram Nature	Motility
Gram Negative cocci shaped	Non Motile

- Biochemical Testing:

Table No 15: Biochemical Testing of C3

Test	Results	Gas
Sucrose	Negative	Negative
Galactose	Positive	Negative
Lactose	Positive	Negative
Xylose	Negative	Negative
Glucose	Positive	Negative
Catalase	Positive	-
H ₂ S	Postive	-

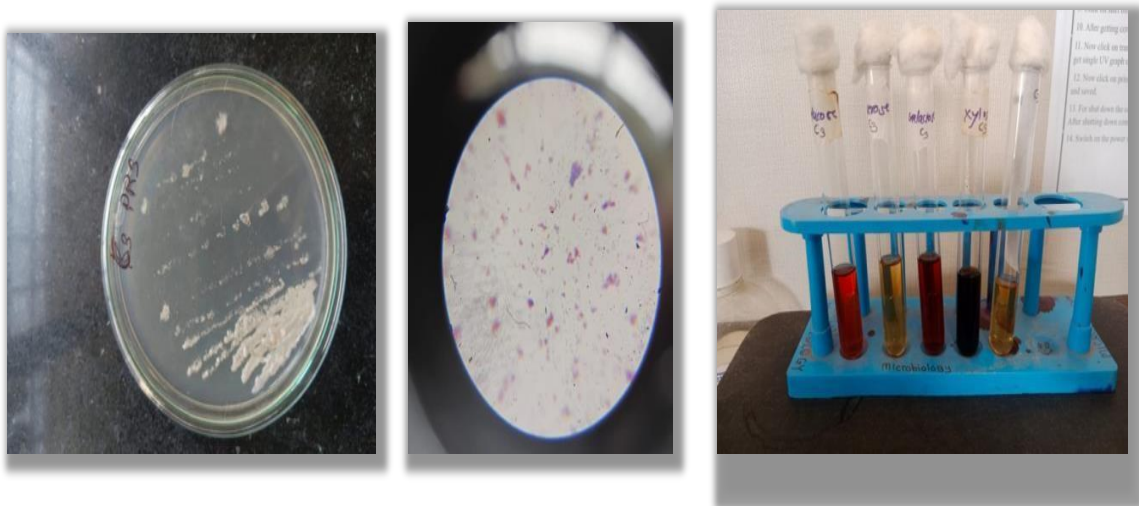


Fig No. 14: Morphological (colony and microscopic) and biochemical characterization of the isolated bacterial strain C3.

Discussion:

The five isolates (K1, K2, C1, C2, C3) showed morphological and biochemical diversity, indicating a mixed microbial population in kombucha culture. Differences in Gram nature and sugar utilization confirm varied metabolic activity among isolates. This diversity plays an important role in fermentation and cellulose production.

5. Confirmation of Acid Producing Bacteria:

Out of the five isolates, some showed the formation of clear zones around the colonies on GYC medium, indicating acid production due to the dissolution of calcium carbonate. The remaining isolates did not show any clear zones were considered nonacid producing.



Fig No 15: Colony characteristics of isolates cultured on GYC agar

Table no 16: Observation Table

Isolate No	Colony observation on GYC medium	Clear Zone
K1	Growth Observed	Present
K2	Growth Observed	Present
C1	Growth Observed	Present
C2	Growth Observed	Present
C3	Growth Observed	Absent

Discussion:

Clear zone formation around some colonies on GYC medium indicates acid production and confirms their ability to metabolize sugars. Isolates without clear zones were non-acid producers. This helps differentiate active cellulose-associated bacteria from non-functional strains.

6. Harvesting of Bacterial Cellulose Layer:

The collected layer appeared as a thick gelatinous sheet. The cellulose layer was washed with distilled water to remove residual tea components. After washing and alkaline treatment, the cellulose becomes clean, flexible and translucent.



Fig No. 16: Before treatment



Fig No.17: After treatment

Discussion:

The harvested layer appeared thick and gelatinous, indicating successful cellulose formation. Washing and alkaline treatment removed impurities, resulting in clean, flexible, and translucent bacterial cellulose, confirming its purity and quality.

7. Bacterial Cellulose Confirmation Tests:

7.1. Acid Hydrolysis Test:

The bacterial cellulose sample, upon treatment with dilute sulfuric acid followed by neutralization, was subjected to Benedict's test. A color change to blue to green precipitate was observed, indicating the presence of reducing sugars. This confirms the hydrolysis of cellulose into glucose units.

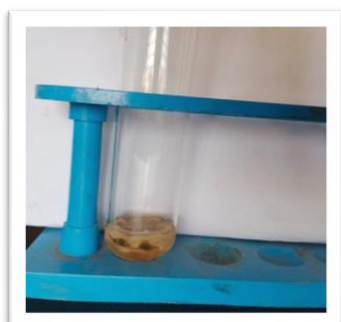


Fig No. 18: Before Treatment



Fig No. 19: After Treatment

7.2. Schultze's Reagent Test:

When iodine solution was added to the bacterial cellulose sample, brown colour is observed. After addition of zinc chloride solution blue color is observed indicating the positive test.

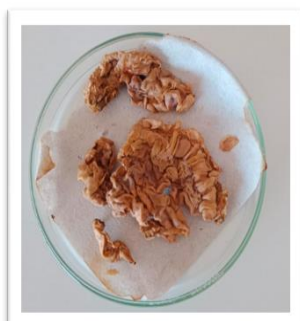


Fig No. 20: Before any addition



Fig No. 21: After addition of Iodine



Fig No. 22: After addition Zinc chloride solution

Table No 17: Test Results of Cellulose confirmation tests

Test	Observation	Conclusion
Acid Hydrolysis Test	Green color observed	Cellulose present
Iodine Test	Blue color observed	Cellulose Present

Discussion:

The acid hydrolysis test indicated the breakdown of cellulose into reducing sugars, confirming the presence of cellulose. The Schulze's Reagent test showed no blue color, indicating absence of starch and supporting the purity of cellulose. Overall, these results confirm successful production and identification of bacterial cellulose.

A decorative horizontal scroll graphic with a light gray background and a dark gray border. The scroll is unrolled in the center, with the word "Conclusion" written in a large, bold, black serif font. The left and right ends of the scroll are rolled up, showing a dark gray inner layer.

Conclusion

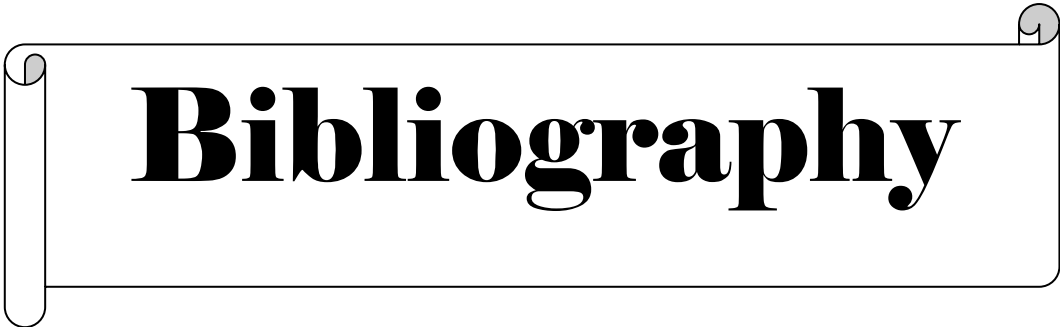
Conclusion:

The present study successfully demonstrated that Kombucha SCOBY can serve as an effective and sustainable source for the production of bacterial cellulose. During fermentation, a gradual formation of a cellulose pellicle was observed, which increased in thickness over time, confirming active microbial synthesis under suitable conditions. The decrease in pH and development of acidic characteristics further indicated proper fermentation activity.

Isolation and characterization of microflora revealed the presence of multiple bacterial strains, among which several showed acid-producing ability, confirming their role in fermentation. The harvested cellulose appeared as a thick, gelatinous, and flexible layer, which became cleaner and more translucent after alkali treatment, indicating successful purification.

Confirmation tests supported the presence of cellulose: the acid hydrolysis test showed positive results with the formation of reducing sugars, validating the breakdown of cellulose into glucose units. The Schultze's Reagent Test indicated the absence of starch, although slight variation in observations suggests the need for careful interpretation.

Overall, the study confirms that Kombucha-derived bacterial cellulose is a pure, biodegradable, and efficient biopolymer with potential applications in various industrial, food, and biomedical fields. The results highlight its feasibility as an eco-friendly alternative to plant-based cellulose.



Bibliography

Bibliography :

1. Iguchi, M., Yamanaka, S., & Budhiono, A. (2000). Bacterial cellulose—a masterpiece of nature’s arts. *Journal of Materials Science*, 35, 261–270.
2. Kontturi, K. S., Lee, K.-Y., & Kontturi, E. (2021). Influence of biological origin on the tensile properties of cellulose nanopapers. *Cellulose*, 28(10), 6619–6628.
3. Lupascu, R. E., Ghica, M. V., Dinu-Pirvu, C. E., Popa, L., Velescu, B. S., & Arsene, A. L. (2021). An overview regarding microbial aspects of production and applications of bacterial cellulose. *Applied Microbiology and Materials*, 15, 676.
4. Rol, F., Belgacem, M. N., Gandini, A., & Bras, J. (2019). Recent advances in surfacemodified cellulose nanofibrils. *Progress in Polymer Science*, 88, 241–264.
5. Stanisławska, A., Staroszczyk, H., & Szkodo, M. (2020). The effect of dehydration/rehydration of bacterial nanocellulose on its tensile strength and physicochemical properties. *Carbohydrate Polymers*, 236, 116023.
6. Reiss, J. (1994). Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 198(3), 258–261.
7. Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 538–550.
8. Marsh, A. J., O’Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology*, 38, 171–178.
9. Reva, O. N., Zaets, I. E., Ovcharenko, L. P., Kukhareno, O. E., & Shpylova, S. P. (2015). Metabarcoding of kombucha microbial community grown in different microenvironments. *AIMS Microbiology*, 1(2), 68–83.

10. Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., & Taillandier, P. (2018). Understanding Kombucha tea fermentation: A review. *Journal of Food Science*, 83(3), 580–588.
11. Rajwade, J. M., Paknikar, K. M., & Kumbhar, J. V. (2015). Applications of bacterial cellulose and its composites in biomedicine. *Applied Microbiology and Biotechnology*, 99(6), 2491–2511.
12. Choi, S. M., Shin, E. J., & Kim, S. H. (2022). Recent advances in bacterial cellulose: Structure, biosynthesis, and applications. *Carbohydrate Polymers*, 290, 119481.
13. Barja, F. (2021). Bacterial nanocellulose production and biomedical applications. *Journal of Biomedical Research*, 35(4), 310–317.
14. Römling, U. (2015). Bacterial cellulose biosynthesis: Diversity of operons, subunits, products, and functions. *Trends in Microbiology*, 23(9), 545–557.
15. Zhong, C. (2020). Industrial-scale production and applications of bacterial cellulose. *Frontiers in Bioengineering and Biotechnology*, 8, 605374.
16. Malcı, K. (2024). Engineering bacterial cellulose with synthetic biology. *ACS Synthetic Biology*, 13(12), 3857–3875.
17. Wang, J., Tavakoli, J., & Tang, Y. (2019). Bacterial cellulose: Production, properties, and applications. *Cellulose*, 26(8), 4091–4114.
18. Lahiri, D., Nag, M., Dutta, B., Mukherjee, D., & Ray, R. R. (2021). Bacterial cellulose: 000Production, characterization, and applications. *International Journal of Biological Macromolecules*, 166, 1044–1061.
19. Manuel, R. N., Bera, S., Saha, S., & Bhattacharya, P. (2020). Plant- vs. bacterial-derived cellulose for wound healing: A review. *International Journal of Biological Macromolecules*, 164, 2088–2101.
20. Girard, V. D., McKenna, J. D., & Yates, M. D. (2024). Bacterial cellulose: A comprehensive review. *Journal of Applied Polymer Science*, 141(12), e54012.

21. Popa, L., Trică, B., Dinescu, S., Costache, M., & Sima, L. E. (2022). Bacterial cellulose — A remarkable polymer as a source for innovative materials. *International Journal of Molecular Sciences*, 23(3), 1430.
22. Naomi, R., Bahari, H., Ridzuan, P. M., Othman, F., & Baharin, M. F. (2020). Plant- vs. bacterial-derived cellulose for wound healing: A review. *International Journal of Biological Macromolecules*, 164, 1037–1051.
23. Chawla, P. R., Bajaj, I. B., Survase, S. A., & Singhal, R. S. (2009). Microbial cellulose: Fermentative production and applications. *Food Technology and Biotechnology*, 47(2), 107– 124.
24. Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., & Dorris, A. (2011). Nanocelluloses: A new family of nature-based materials. *Angewandte Chemie International Edition*, 50(24), 5438–5466.
25. Yoshinaga, F., Tonouchi, N., & Watanabe, K. (1997). Research progress in biopolymer cellulose produced by *Acetobacter xylinum*. *FEMS Microbiology Reviews*, 16(4), 279–286.
26. Yamanaka, S., Watanabe, K., Kitamura, N., Iguchi, M., Mitsunashi, S., Nishi, Y., & Uryu, M. (1989). The structure and mechanical properties of sheets prepared from bacterial cellulose. *Journal of Materials Science*, 24, 3141–3145.
27. Ross, P., Mayer, R., & Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiological Reviews*, 55(1), 35–58.
28. <https://www.toyokombucha.com>
29. Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health: A review. *Food Research International*, 33(6), 409–421.
30. Greenwalt, C. J., Ledford, R. A., & Steinkraus, K. H. (2000). Determination and characterization of the antimicrobial activity of the fermented tea Kombucha. *LWT-Food Science and Technology*, 33(1), 13–19.
31. Gagliardi, T. R., et al. (2025). Kombucha Bacterial Cellulose: A Promising Biopolymer for

Sustainable Applications. *Cellulose*, 32(4), 2175–2190.

32 .Lin, S. P., Huang, Y. H., Hsu, K. D., Chen, Y. K., & Cheng, K. C. (2019). Bacterial cellulose-based flexible supercapacitor with high energy density. *Cellulose*, 26(12), 6973– 6985.

33 .Singh, P., Patel, R., & Tripathi, R. (2023). Sustainable bacterial cellulose production from fruit peel waste for biopolymer applications. *Bioresource Technology Reports*, 22, 101406.

34 .Chawla, P. R., Bajaj, I. B., Survase, S. A., & Singhal, R. S. (2022). Factors influencing bacterial cellulose production and quality. *Food Reviews International*, 38(3), 524–542.

35.Zhong,C. (2020). Industrial-scale production and applications of bacterial cellulose.

Frontiers in Bioengineering and Biotechnology, 8, 605374.

36 .Gupta, S., Sharma, A., & Chawla, P. (2023). Kombucha-derived bacterial cellulose:

Variability and process control challenges. *LWT–Food Science and Technology*, 180, 114768.

37.Sulaeva, I., Henniges, U., Rosenau, T., & Potthast, A. (2020). Bacterial cellulose: Sustainable production and application. *Cellulose*, 27(9), 5353–5381.

38.Torres, F. G., Commeaux, S., & Troncoso, O. P. (2021). Bacterial cellulose and its applications: A review. *International Journal of Biological Macromolecules*, 177, 258–270.

39 .Bhat, A. H., Khan, I., & Nayak, V. (2023). Quality control and standardization of bacterial cellulose: Current gaps and future perspectives. *Trends in Food Science & Technology*, 137, 364–377.

40. Cai, Z., Chen, Y., Ma, M., & Li, S. (2022). Eco-friendly purification of bacterial cellulose using enzymatic and ultrasonic methods. *Carbohydrate Polymers*, 283, 119168.
41. Nelson, D. L., & Cox, M. M. (2017). *Lehninger principles of biochemistry* (7th ed.). W.H. Freeman and Company.
42. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428. <https://doi.org/10.1021/ac60147a030>
43. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.
44. Sadasivam, S., & Manickam, A. (2008). *Biochemical methods* (3rd ed.). New Age International Publishers. Nelson, D. L., & Cox, M. M. (2017). *Lehninger principles of biochemistry* (7th ed.).